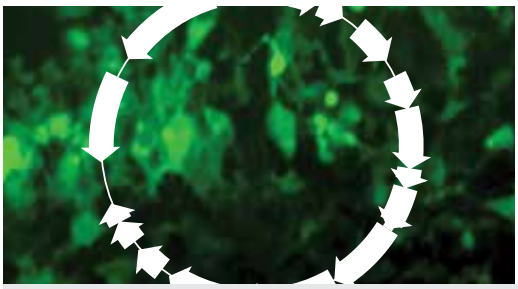
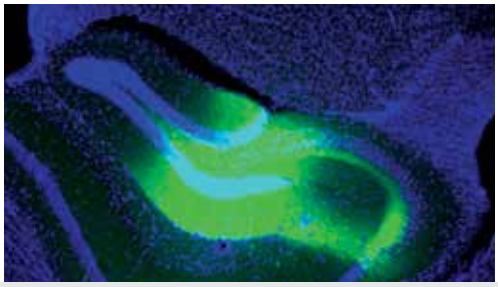
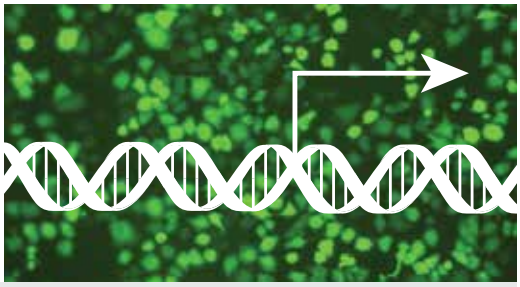
GENE DELIVERY & EXPRESSION

GENE DELIVERY & EXPRESSION

LENTIVIRUS, PIGGYBAC, MINICIRCLES, AND MORE





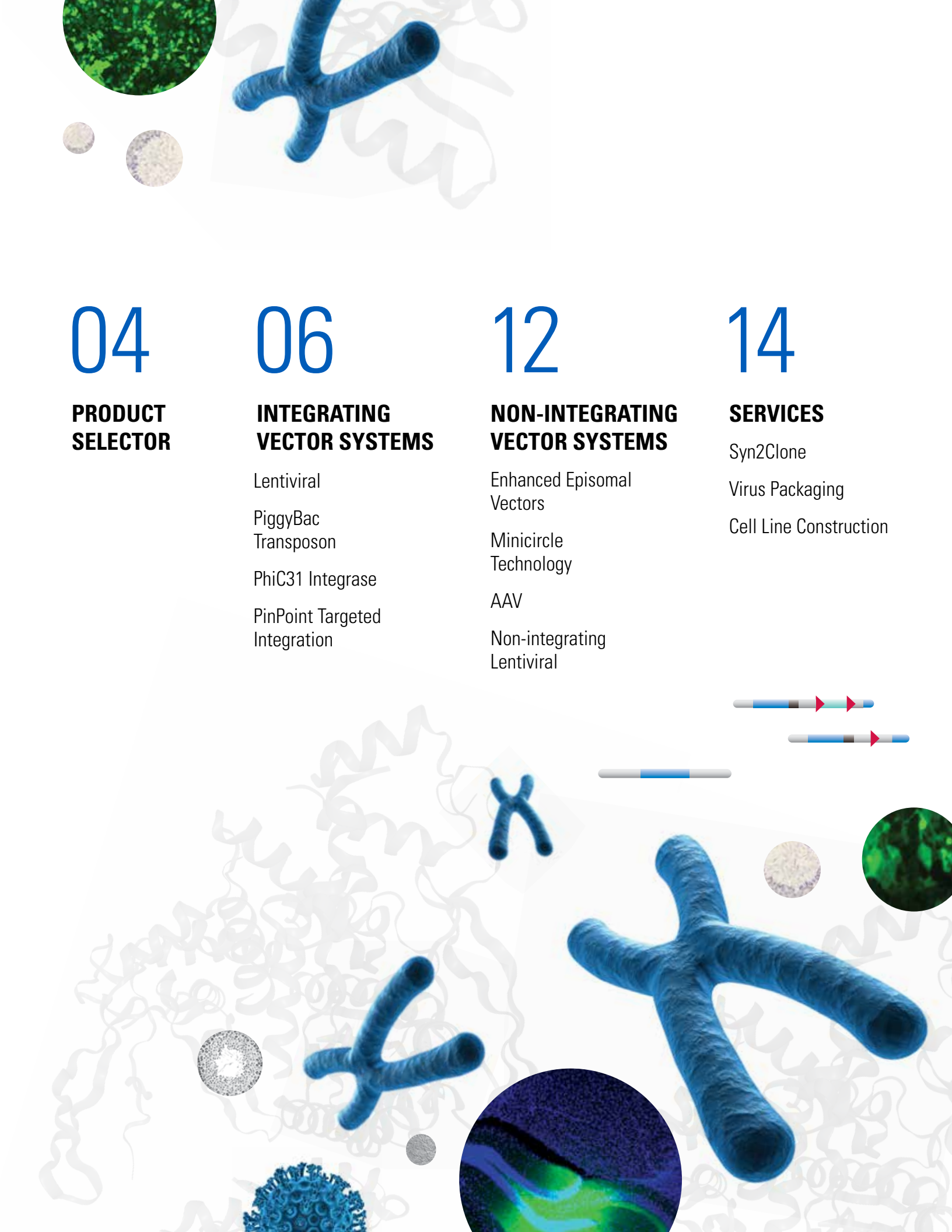


SYSTEMBIO.COM

SBI System Biosciences
Harnessing innovation to drive discoveries

SYSTEMS FOR ANY APPLICATION: LENTIVIRAL TOOLS AND MORE

In today's busy labs, getting experiments to work right the first time is critical—not only do you want results you can trust, you want them fast. Which is why SBI has spent over a decade developing a range of exceptionally high-performing products for gene delivery and expression. From our popular high-titer lentivirus production tools to the latest in AAV-based gene delivery systems and a range of reliable integrating and non-integrating gene expression vectors, SBI delivers high-quality, cutting-edge products and services for mammalian gene delivery and expression. With products and services that have been used in thousands of peer-reviewed papers, SBI is your trusted partner for high-quality research.



A WEALTH OF OPTIONS FOR GENE DELIVERY AND EXPRESSION

Integrating Vectors

With integrating vectors, the vector DNA becomes permanently incorporated into the genome, often at multiple copy numbers. Depending on the system, integration can be random or directed to a specific locus.

USES: Integrating vectors are great for general gene or non-coding RNA functional studies, and applications where heritable expression is desired, such as cell line construction.

VIRAL SYSTEMS

For researchers who wish to take advantage of high-efficiency viral transduction to introduce their gene-of-interest into target cells.

06 Lentivectors
HIGH TITER AND RELIABLE

- RANDOM INTEGRATION
- LIMITED INSERT SIZE
- HIGH COPY NUMBER

NON-VIRAL SYSTEMS

For researchers who want to avoid integrating viral sequences, want to control copy number, or are targeting easy-to-transfect cells.

10 PiggyBac™ Transposon System
EASY, CONSISTENT TRANSGENESIS

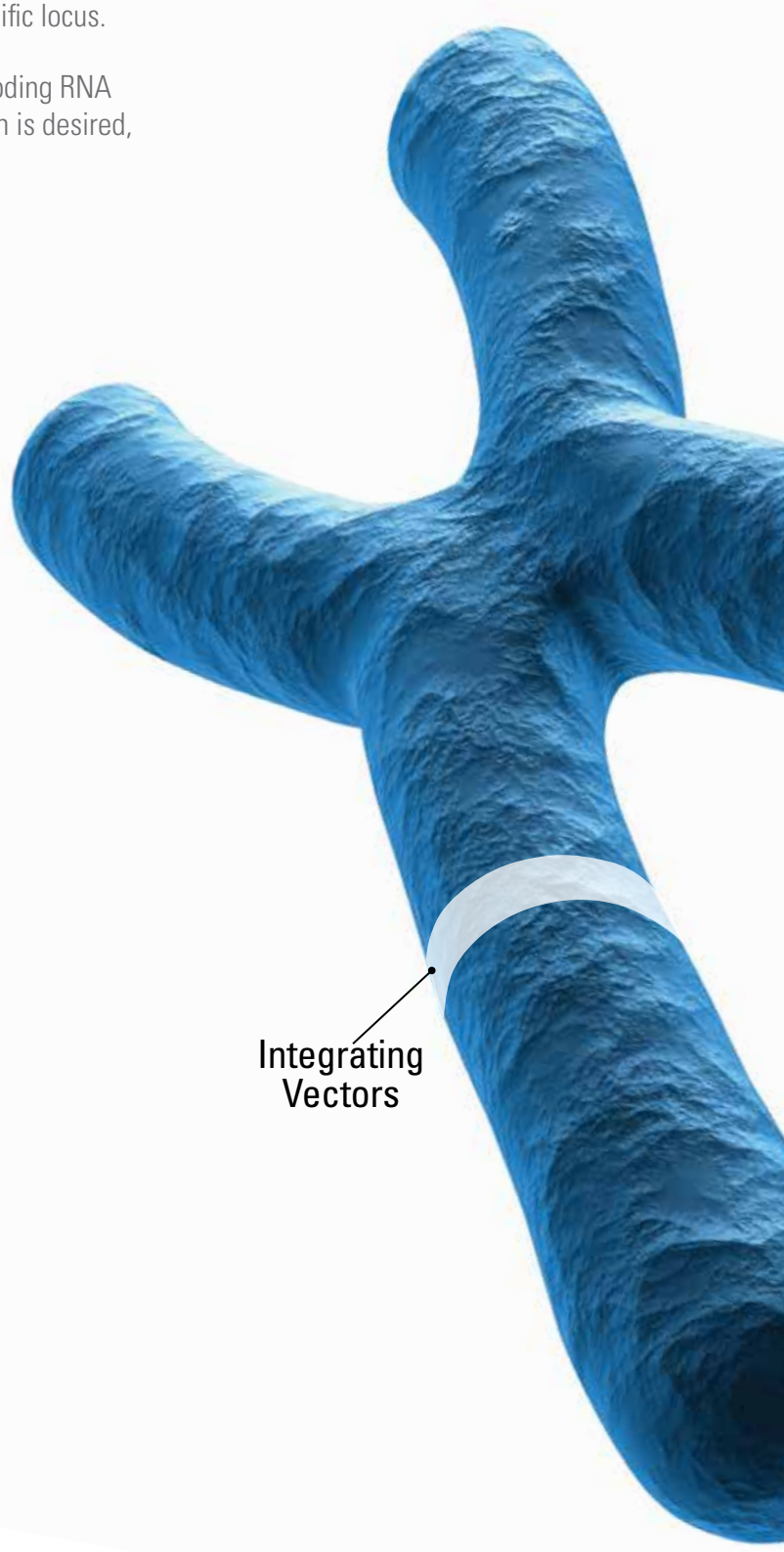
- RANDOM INTEGRATION
- UNLIMITED INSERT SIZE
- HIGH COPY NUMBER

11 PhiC31 Integrase System
ONE-STEP SINGLE-COPY INTEGRATION

- SITE-SPECIFIC INTEGRATION
- UNLIMITED INSERT SIZE
- SINGLE COPY NUMBER

11 PinPoint Targeted Integration System
EXCELLENT FOR CREATING ISOGENIC CELL LINES

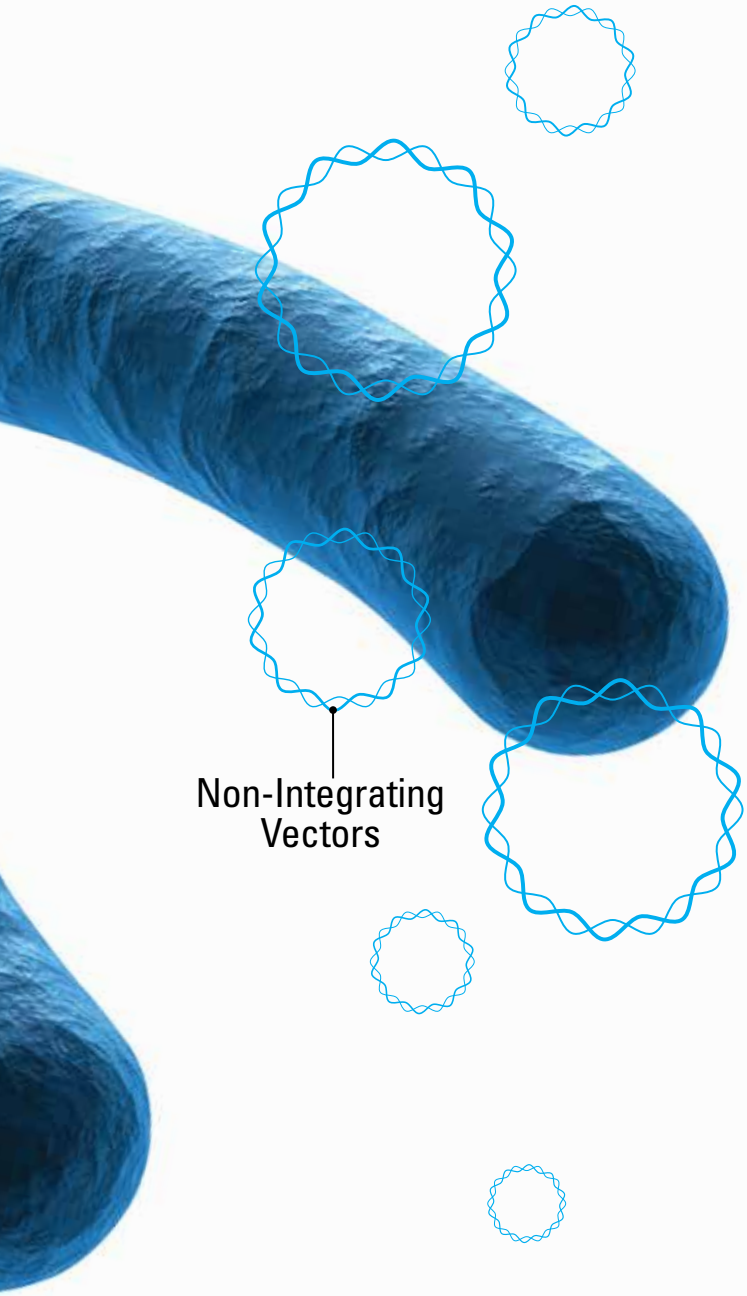
- SITE-SPECIFIC INTEGRATION
- UNLIMITED INSERT SIZE
- SINGLE COPY NUMBER



Non-integrating Vectors

With non-integrating vectors, the vector DNA is maintained episomally, *i.e.*, as an extra-genomic plasmid. All of SBI's non-integrating vector systems provide sustained expression that can last weeks to months in both quiescent and dividing cells.

USES: Non-integrating vectors offer gene expression without the potential for disruption of host gene activity via integration, making them great for sensitive applications such as gene therapy development. SBI's non-integrating vector systems are also an excellent option for long term gene expression when genomic integration must be avoided.



VIRAL SYSTEMS

For researchers who wish to take advantage of high-efficiency viral transduction for non-integrating gene expression.

13 AAV Vectors

EFFECTIVE GENE DELIVERY WITHOUT VIRAL INTEGRATION

- LIMITED INSERT SIZE
- HIGH COPY NUMBER

13 Non-integrating Lentiviral Systems

ALL THE LENTIVIRUS BENEFITS WITHOUT INTEGRATION

- LIMITED INSERT SIZE
- HIGH COPY NUMBER

NON-VIRAL SYSTEMS

Episomal options with virtually no limits on insert size.

12 Minicircle Technology

EPISOMAL EXPRESSION FREE OF FOREIGN DNA

- UNLIMITED INSERT SIZE
- HIGH COPY NUMBER

12 Enhanced Episomal Vectors

EASY EPISOMAL EXPRESSION

- UNLIMITED INSERT SIZE
- HIGH COPY NUMBER

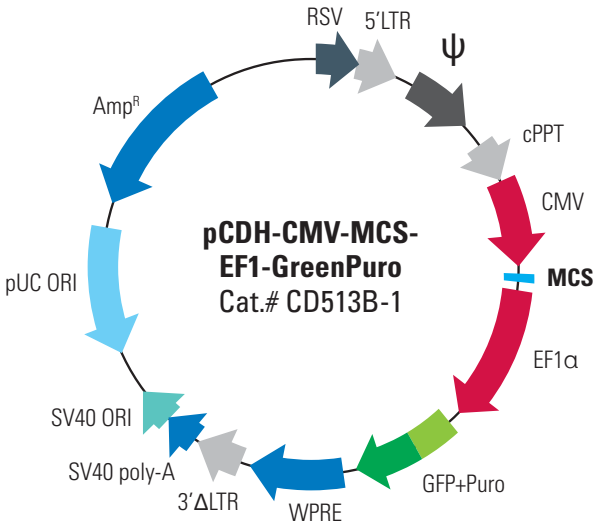
POWERFUL AND FLEXIBLE LENTIVECTORS

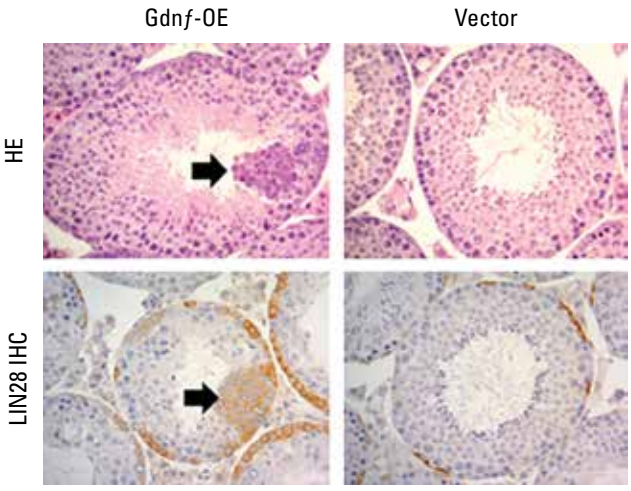
When you want to stably express a gene or non-coding RNA in either dividing cells or quiescent cells, SBI's lentiviral vectors are an excellent choice. Well-regarded in the industry for high, reliable gene expression, our lentiviral vectors come in a variety of formats that support a wide range of different applications.

- Multiple promoter options
- Multiple marker options—Puro, Neo, Hygro, GFP, or RFP
- Coordinated co-expression via T2A or IRES
- Single promoter, dual promoter, and bidirectional promoter formats
- High expression in most hematopoietic, embryonic, and stem cells via the MSCV CpG-deficient promoter

Promoter	Expression Level	Applications
CMV	High	Commonly used in most cell lines (HeLa, HEK293, HT1080)
MSCV	High	Hematopoietic and stem cells
EF1	Medium	Most cell types, including primary cells and stem cells
PGK	Medium	Most cell types, including primary cells and stem cells
UbC	Low	Most cell types, including primary cells and stem cells

*"Over a ten year period, we have worked with many different lentivector backbones. SBI's pGreenPuro shRNA lentivectors provide us with top transduction efficiencies in difficult-to-transduce cells, including primary human CD34+ hematopoietic progenitors." —**Dr. Don M. Wojchowski, Maine Medical Center Research Institute***





Overexpression of mouse GDNF cDNA in six-week old C57BL/6 mouse testes leads to significant proliferation and accumulation of LIN28A positive germ cells. Testes were isolated from mice exposed to lentivirus particles containing either GDNF cloned into SBI's pCDH-EF1-MCS-T2A-Puro vector (Cat# CD527A-1) or vector alone, and then stained using HE or an antibody specific for LIN28A, (from Wei, X. et al., Sci Rep. 2016; 6: 36779. PMCID: PMC5101510).

Tightly-controlled inducible gene expression

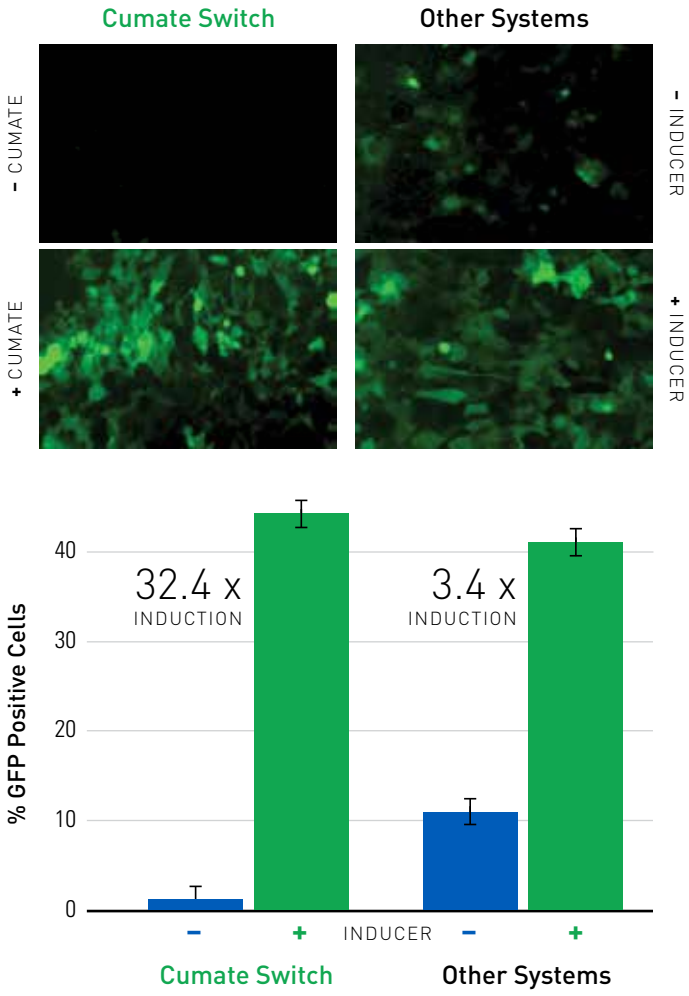
Get robust, titratable gene expression with low background using our SparQ™ cumate-inducible vectors. These vectors take advantage of CymR, a repressor that binds to cumate operator sequences (CuO) with high affinity in the absence of cumate, a non-toxic small molecule. Providing much lower background expression than similar systems, SparQ vectors can provide up to 32-fold induction of gene expression.

- **Robust**—increase expression up to 32-fold
- **Adjustable**—tune expression levels by titrating the amount of cumate
- **Reversible**—turn expression on, then off, then on again
- **Versatile**—choose from all-in-one formats that co-express CymR and your gene-of-interest, or two-vector systems where CymR is expressed from a different plasmid
- **Powerful**—suitable for in vivo applications

See all our SparQ vectors—visit: ***systembio.com/sparq***

See how widely cited SBI's lentiviral vectors are—visit: ***systembio.com/cdna-lentivector-citations***

Need help choosing a lentivector? Contact us at ***tech@systembio.com***



SparQ vectors deliver robust induction with much lower background than other systems.

CHOOSING A VECTOR

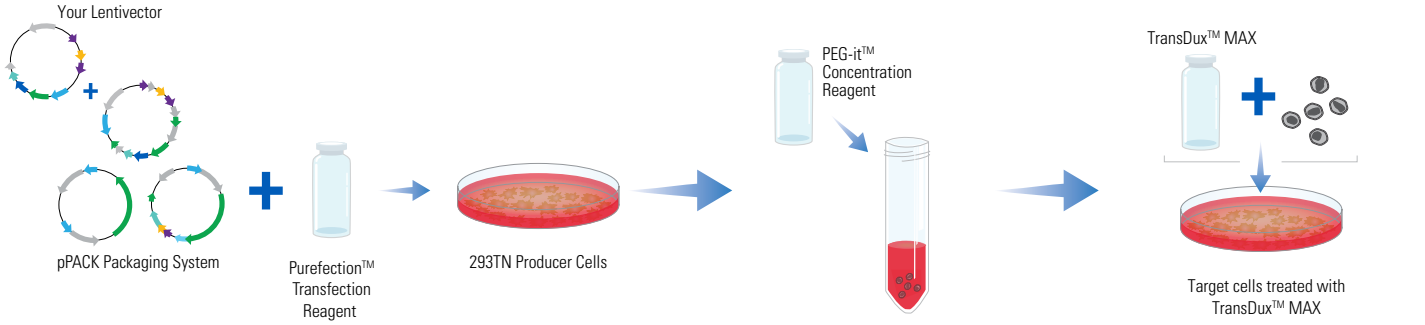
Vector Format	Description	Benefit
Single promoter 	One promoter drives your gene-of-interest Select vectors with T2A or IRES elements for co-ordinated expression of a marker with your transgene	**Efficient**—compact size enables high virus titers
Dual promoter 	Two promoters—one drives your gene-of-interest, the other drives one or more markers	**Convenient**—express transgene and marker(s) via a single vector
Bidirectional promoter 	Two divergent promoters—one drives your gene-of-interest, the other drives one or more markers	**Robust**—avoids low expression due to promoter interference **Convenient**—express transgene and marker(s) via a single vector

For a complete listing of our lentiviral vectors—including vectors for expressing cDNA, miRNA, or shRNA—visit ***systembio.com/lentiviral-expression-vectors***

HIGH PERFORMANCE LENTIVIRAL PRODUCTION AND TRANSDUCTION

Find everything you need to efficiently deliver your lentivector of choice into target cells, from packaging to transduction.

Lentiviral Packaging Workflow



STEP 1
Transfect the packaging system and your lentivector into producer cells.

STEP 2
Collect the cell culture medium and concentrate the pseudoviral particles.

STEP 3
Add virus mixed with TransDux™ MAX to target cells.

"We used the ultra-high titer lentivirus system from SBI to overexpress and/or knockdown miR-33 in vivo in C57BL6 mice. The virus performed very well, easily infecting the liver (our tissue of interest) with high efficiency and little to no observed toxicity. This system allowed us to rapidly and easily address whether miR-33 was playing a physiological role in regulating HDL in vivo."
—Katey Rayner, NYU Langone Medical Center

High titer lentivirus packaging systems

Our highly-regarded pPACK Packaging System (Cat.# LV500A-1) is optimized for producing high titer VSV-G pseudotyped lentiviral particles from any third generation, HIV-based lentivector. The VSV-G pseudotype ensures efficient transduction into a broad range of cell types, including dividing and quiescent cells, primary cells, stem cells, neuronal cells, endothelial cells, and more. Our optimized formula and extensive validation ensure that each batch consistently delivers extremely high titers.

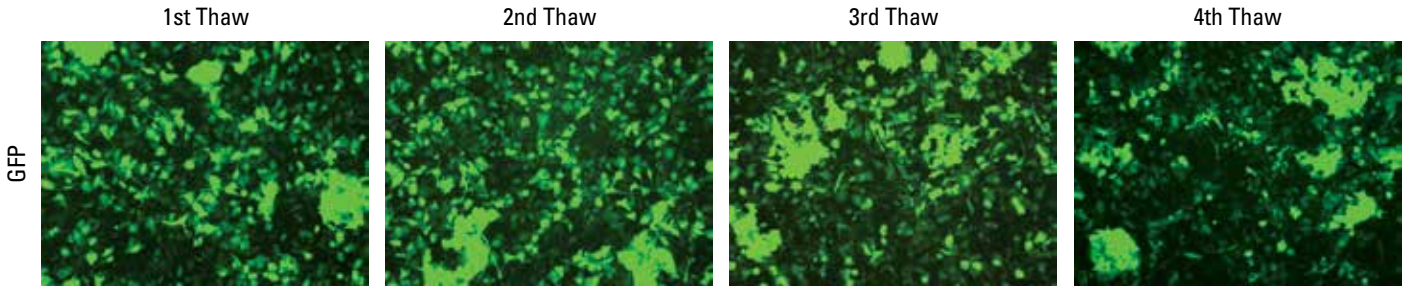
Transfect pPACK into our 293TN producer cell line (Cat.# LV900A-1) using SBI's Purefection Transfection reagent (Cat.# LV750A-1) for optimal production of pseudoviral particles.

New to lentiviral systems? Get up and running fast with either of our Lentiviral kits. The NextGen LentiStarter Kit (Cat.# LV060A-1) includes the pPACK Packaging System, PureFection Transfection Reagent, PEG-it Concentration Reagent, and TransDux™ MAX Transduction Reagent. The NextGen LentiSuite™ Kit (Cat.# LV350A-1) contains the 293TN Producer Cells, pPACK, PureFection, PEG-it, TransDux MAX, and the Global UltraRapid Titering Kit.

PEG-it™ virus precipitation & cryopreservation (Cat.# LV810A-1)

Used in over three-hundred citations, PEG-it enables easy concentration of pseudoviral particles to produce ultra-high titers. Concentrate pseudoviral particles even from large volumes of medium by removing the need for ultracentrifugation. Simply add PEG-it to the collected medium, incubate overnight at 4°C, and spin at 1500*g* for 30 minutes.

In addition, PEG-it acts as a cryopreservative for concentrated virus. Lentivirus concentrated with PEG-it lasts longer in the freezer and survives multiple freeze-thaw cycles with minimal loss of titer.



Lentivirus concentrated with PEG-it retains high titers even after four freeze-thaw cycles. HT1080 Cells transduced with LV605VA-1 after several freeze-thaw cycles.

TransDux™ MAX Transduction Enhancer Reagent (Cat.# LV860A-1)

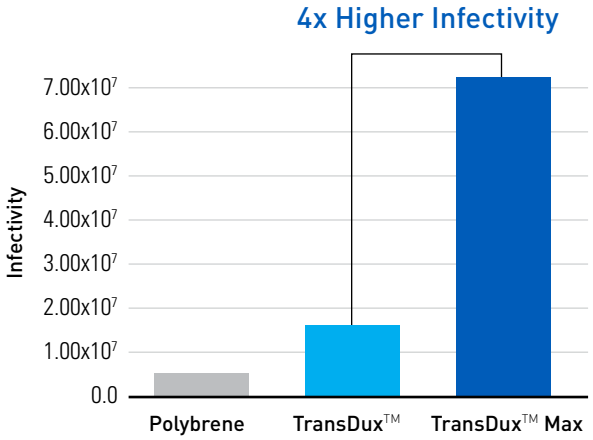
Maximize gene delivery with TransDux™ MAX. Delivering even higher transduction efficiencies than both polybrene and the original TransDux™ reagent and lower toxicity than polybrene in all tested cell lines, TransDux™ MAX works with all types of packaged lentivirus and requires minimal hands-on time.

Positive transduction control viruses

Optimize transduction conditions or determine the sensitivity of your target cells to infection with our ready-to-go packaged positive transduction controls. Transduced cells stably express copepod green fluorescent protein (copGFP) and RFP from your choice of promoters.

Global UltraRapid™ Titering Kit (Cat.# LV961A-1)

Quickly and accurately measure your viral titer using our qPCR-based Global UltraRapid Titering Kit. Go from infected cells to titer determination in less than 3 hours, with no isolation or concentration of genomic DNA required.



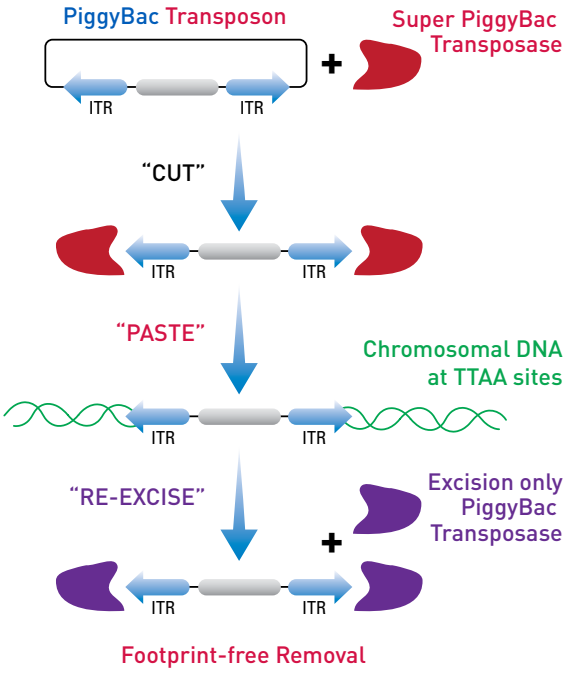
TransDux™ MAX delivers higher transduction efficiencies than both polybrene and the original TransDux™ reagent.

Learn more about SBI's lentiviral technologies—visit:
systembio.com/lentiviral-technology

Watch our lentiviral packaging video tutorial—visit:
systembio.com/jove

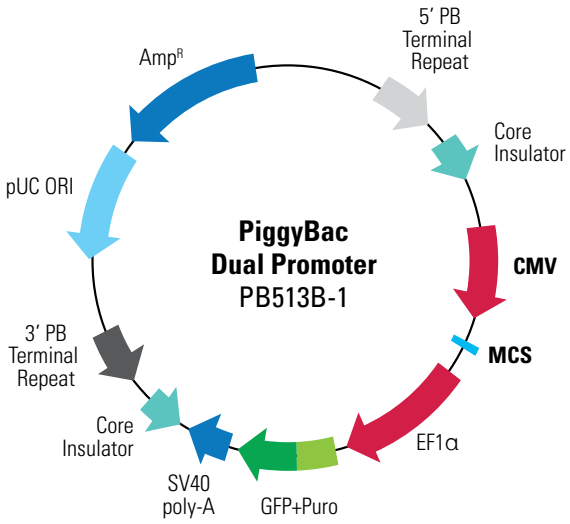
See just how widely-cited SBI's lentiviral products are—visit:
systembio.com/lentiviral-production-citations

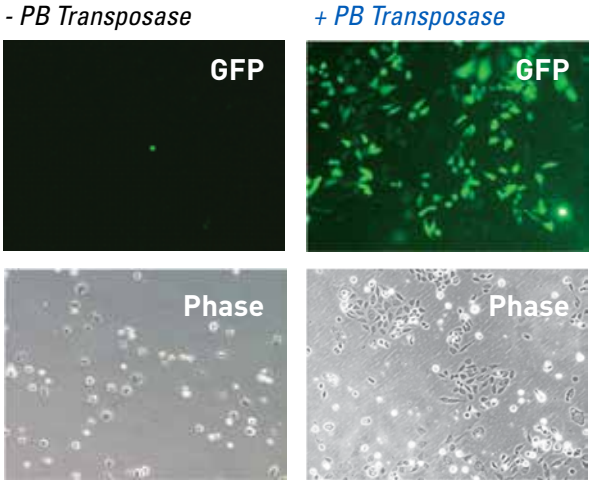
EASY, CONSISTENT TRANSGENESIS WITH PIGGYBAC



When you want to create stable cell lines for sustained gene expression and are using easily transfected cells, PiggyBac is a simple, straightforward, and highly consistent technology. Transposon-mediated integration works via a cut-and-paste mechanism to insert your gene-of-interest into the genome. The only vector sequences inserted are those elements flanked by the inverted terminal repeats.

- **Versatile**—no limit on insert size
- **Multiplex-able**—transfect multiple PiggyBac constructs to simultaneously integrate multiple genes at once
- **Inducible**—all-in-one, cumate-inducible PiggyBac system available (see p. 7 for more information on the cumate system)
- **Reversible**—excision-only plasmid delivers footprint-free removal of the insert





Puromycin selection (10 µg/ml) 3 days

Human 293 cells were transfected with the Super PiggyBac transposase transient expression vectors PB210PA-1 and PB513B-1 and cells imaged after seven days. A high proportion of cells were puro-resistant and GFP positive.

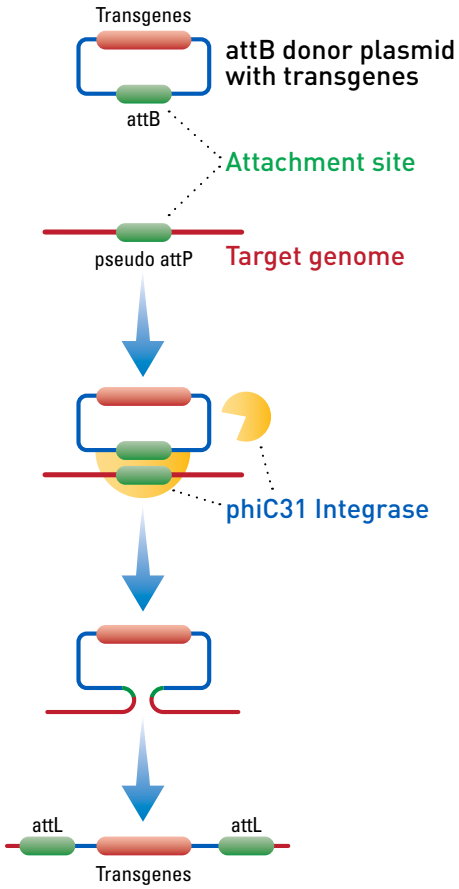
See all the different promoters and vector formats available—visit ***systembio.com/piggybac***

CONTROLLED, SINGLE-COPY INTEGRATION SYSTEMS

The one-step PhiC31 Integrase System

An excellent option for one-step, non-viral gene delivery, the PhiC31 Integrase System enables transgene expression from a single, integrated copy. Based on the bacteriophage PhiC31 integration system that integrates into bacterial attP sites, SBI's PhiC31 Integrase System takes advantage of pseudo-attP sites that occur at low frequency in mammalian genomes, where it inserts the PhiC31 donor vector. The PhiC31 Integrase System can integrate a plasmid of any size and requires no cofactors, for stable, heritable gene expression.

*Learn more about the PhiC31 system and see the full range of vectors—visit **systembio.com/PhiC31***



The two-step PinPoint Targeted Integration System

The PinPoint Targeted Integration system allows users to easily and efficiently create isogenic stable cell lines in mammalian and other cell types, enabling more accurate genotype to phenotype correlations.

The two-step approach involves:

1) Introducing the PinPoint Placement vector into the target cells.
Placement vector is transfected into target cells, and then integrated into the genome using either the PhiC31 Integration System or an HR Donor vector targeting any site of interest, such as the AAVS1 Safe Harbor site. This cell line can serve as the parent cell line for isogenic strain generation.

2) Introducing the PinPoint Donor vector, which expresses the gene-of-interest, into the cells created in Step 1.
Donor vector is co-transfected into target cells with the vector that expresses the PinPoint Integrase. PinPoint Integrase, which does not recognize any pseudo-attP sites in the human or mouse genomes, mediates rapid and highly specific integration of the donor vector into the PinPoint Placement site.

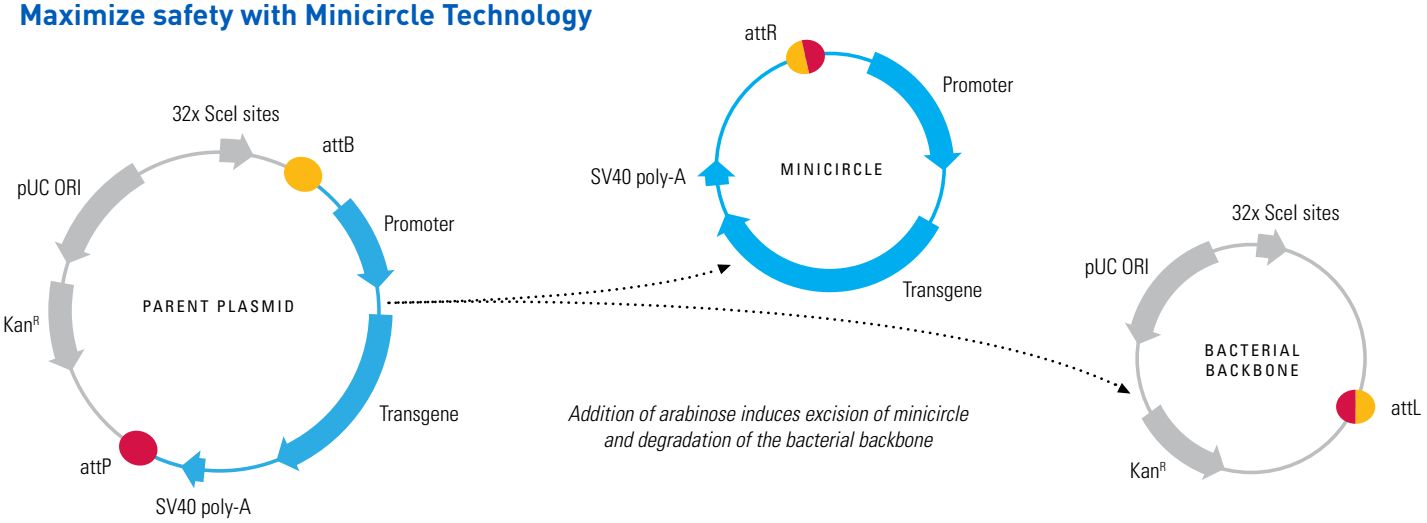
An optional third step can be used to remove the donor vector sequences via Cre/Lox recombination.

*Learn more about the PinPoint system and see the full range of vectors—visit **systembio.com/pinpoint***

NON-INTEGRATING OPTIONS FOR TRANSGENE EXPRESSION

When you want sustained transgene expression without introducing any foreign DNA—such as for model animal and gene therapy development—Minicircle Technology is a great gene expression option. Produced as small excised, circular DNA fragments from a parental plasmid, the non-viral, episomal Minicircle expression cassette is free of any bacterial plasmid DNA sequences, and comes with a variety of promoter and reporter combinations. Their small size facilitates more efficient transfection than what's possible with standard-sized plasmids, and, while Minicircles do not replicate with the host cell, expression lasts for 14 days or longer in dividing cells, and can continue for months in non-dividing cells.

Maximize safety with Minicircle Technology

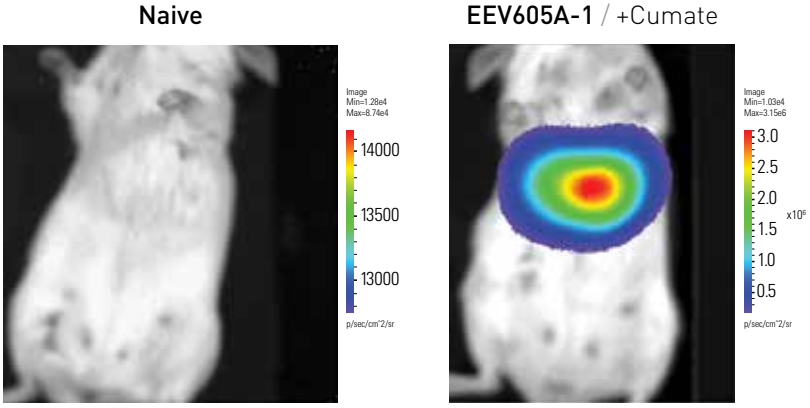


Learn more about Minicircle Technology and see our full range of minicircle vectors—visit ***systembio.com/minicircle-technology***

Sustained episomal expression with Enhanced Episomal Vectors

SBI's Enhanced Episomal Vectors (EEVs) are another excellent choice for non-integrating, non-viral gene expression. Because they replicate in synchrony with the host cell, they are stably inherited and can be used for long-lasting expression without modifying the host genome. Easy-to-produce, SBI's EEVs come with a wide range of promoters and markers, including our cumate-inducible system.

Learn more about EEVs and see our full range of vectors—visit ***systembio.com/EEV***



EEVs deliver robust, transducible gene expression in vivo. To learn more, visit ***systembio.com/eev-in-vivo.***

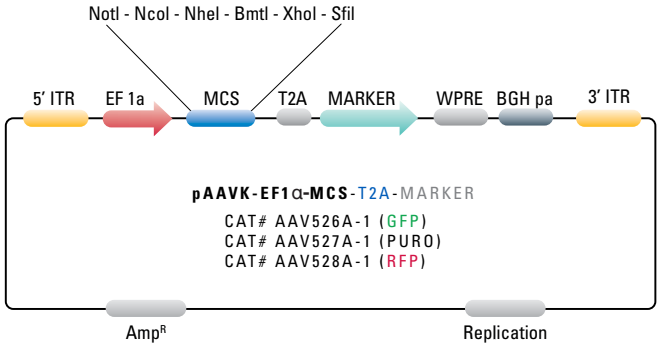
Take advantage of the power of rAAV with SBI's AAVanced Cloning and Expression Vectors

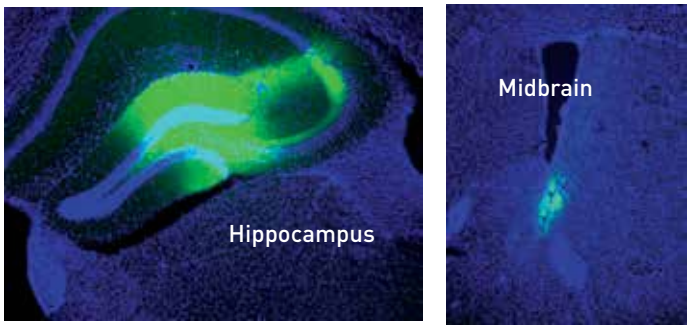
Widely used for gene therapy and genome editing, recombinant Adeno-associated Virus (rAAV) vectors combine the efficacy of viral transduction without vector integration. SBI's AAVanced™ rAAV expression vectors are based on the commonly used AAV2 backbone, and contain inverted terminal repeats (ITRs) at both ends of the DNA with room for an exogenous promoter and open reading frame. To produce a high titer of viral particles, expression and packaging vectors are transiently co-transfected into suitable mammalian virus producer cells (*e.g.* HEK 293T cells) for subsequent isolation of rAAV virus particles from culture media using SBI's **AAVanced Concentration Reagent** (Cat.# AAV100A-1)—no producer cell lysis necessary.

Note that for all AAVanced vectors, the size of the cassette between the ITRs (including insert) must be less than 5 kb to maintain efficient packaging.

AAVanced™ Concentration Reagent enables one-step rAAV particle isolation from packaging cell media—learn more at ***systembio.com/aavanced-concentration***

See the full range of AAVanced vector options—visit ***systembio.com/aavanced-vectors***





Green=GFP fluorescence Blue=DAPI staining

Images of hippocampal and midbrain sections from 6-week old C57 mice injected with AAV virus (ITR-PGK-GFP-ITR) concentrated using AAVanced Concentration Reagent. Images courtesy of Woo-Ping Ge, PhD., University of Texas, Southwestern Medical Center.

Robust lentiviral gene delivery without vector integration

Combining all the advantages of lentiviral vectors with the benefits of episomal expression, the pPACK-ID lentiviral packaging system provides a new way to take advantage of SBI's large portfolio of lentiviral vectors. It's even compatible with your already-made lentiviral expression constructs.

- **Safe**—avoids the risks of insertional mutagenesis
- **Controlled**—transient expression in dividing cells, stable expression in quiescent cells
- **Flexible**—infect a wide range of cells with the VSV-G pseudotyped envelope
- **Optimized**—well-characterized virus production based on SBI's powerful pPACK system
- **Ready-to-use**—fully compatible with all of SBI's third generation lentiviral transfer vectors and all downstream virus concentration methods (PEG-it, ultracentrifugation, ultrafiltration)

Learn more about pPACK-ID—visit ***systembio.com/pPACK-ID***

HIGH QUALITY CUSTOM GENE SYNTHESIS, PACKAGING, AND CELL LINE SERVICES

With a focus on quality and customer service, our expert scientific team is ready to help you streamline your studies with our Gene Delivery and Expression Services.

Experienced team with over a decade of successful gene delivery and expression projects

- Well-versed in the latest techniques
- Well-equipped with SBI's high-quality gene delivery and expression products
- Well-regarded—supports hundreds of projects each year

State-of-the-art facility in Palo Alto, California

- All projects completed on-site
- Ensures consistent quality, confidentiality, and timeliness of delivery

Syn2Clone™ vector construction service

A simpler, easier way to get the constructs you need

With SBI's highly affordable Syn2Clone service, you can order any of SBI's well-validated and highly published vectors with your gene-of-interest already in it. Just imagine, for little more than the cost of the empty vector, we will synthesize your gene-of-interest and clone it directly into the SBI vector of your choice, satisfaction guaranteed. Just send us your sequence and vector selection, and we'll take care of the rest.

Virus packaging

From the industry leader in lentivirus manufacturing

Save time and effort—get ready-to-transduce, high-quality, high-titer lentiviral preparations in as little as ten days. Use your own lentivector construct, or take advantage of our Syn2Clone or Custom Construct services and we'll handle vector construction as well. Large scale production (1–10 ml) also available.

Stable Cell Line Construction

Expert service that's fast and reliable

With a wide range of tools at our disposal—from our outstanding CRISPR/Cas9 reagents to our highly-regarded lentiviral vectors for overexpression, luciferase labeling, and more—we can quickly and easily deliver the cell lines you need to speed your studies.

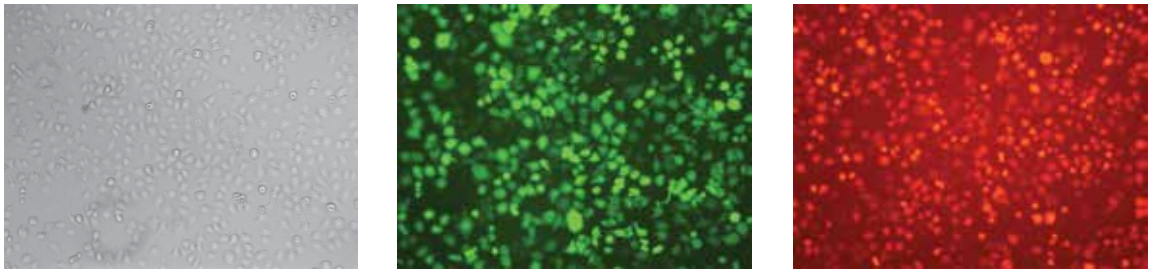
With our Stable Cell Line Construction Service, you get:

- Our expertise in working with a wide variety of cell lines (adherent and suspension)
- Our quality customer service experience, with high success rates, frequent communication, and reliable timelines
- Transduction at high and low MOIs, complimentary control lines, and more with our premium stable cell line service
- PhiC31 & PinPoint technology when non-viral methods are required
- CRISPR/Cas9 technologies for gene knockouts, edits, and knock-ins on the genomic level
- Guaranteed quality assurance, competitive pricing, and fast turn-around time

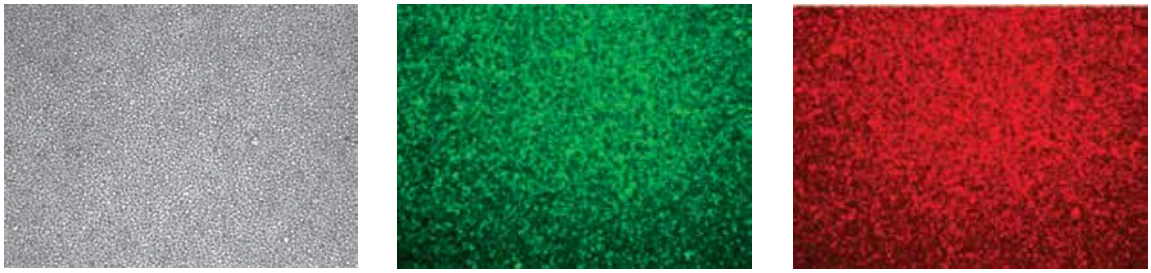
Available Titer Options

Titer	IFU/mL	Applications
Regular Titer	$>10^7$	Standard cell culture models
High Titer	$>10^8$	More difficult-to-transduce cells (e.g. suspension cells)
Ultra-high Titer	$>10^9$	Very difficult-to-transduce cells, such as stem cells and primary cells; in vivo applications

Small Cell Lung Carcinoma Cell line—H2286 (Adherent)

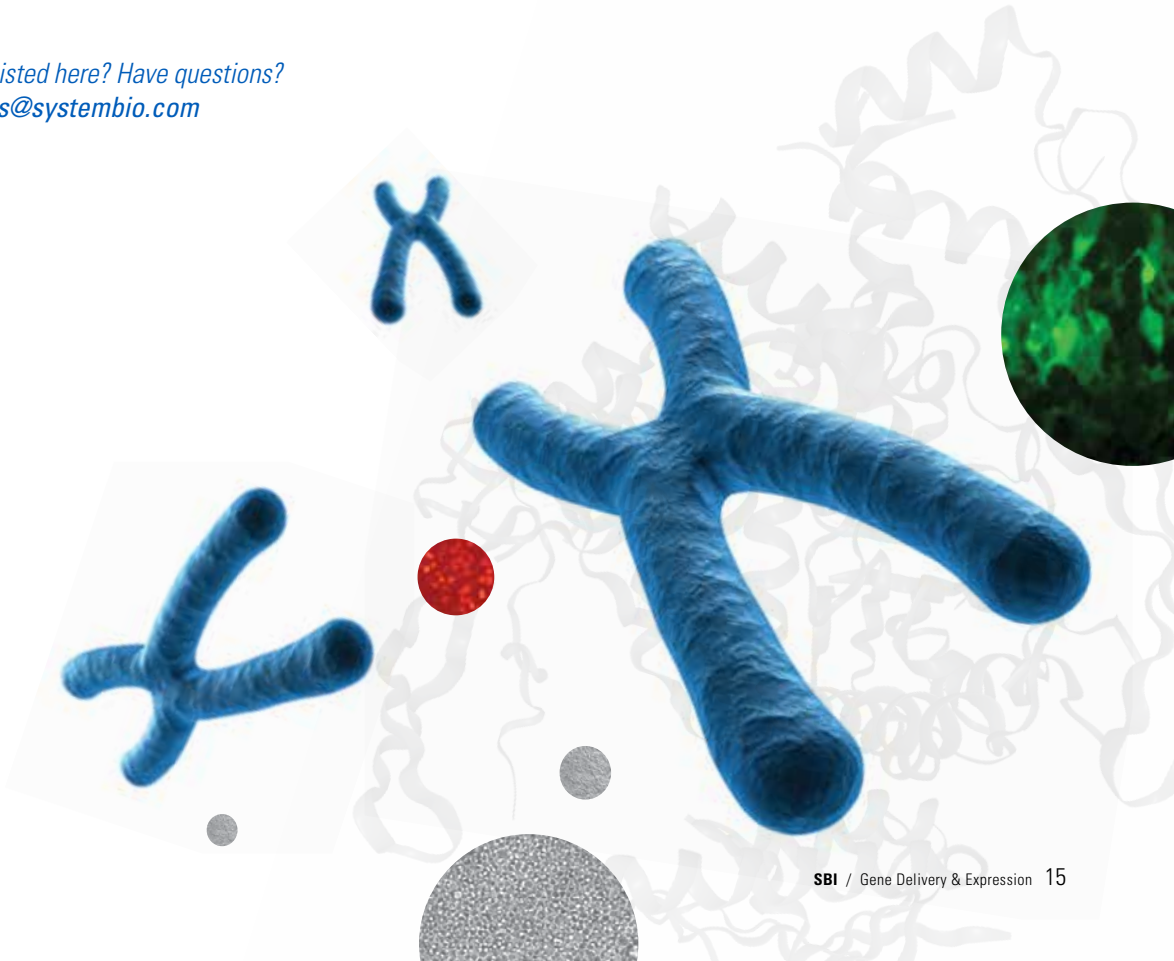


Multiple Myeloma Cell line—H929 (Suspension)



(Top row) Stable cell line expressing two genes-of-interest with GFP, RFP, hygromycin, and puromycin selection markers. H2286 adherent cells were grown under puromycin and hygromycin selection. (Bottom row) Stable cell line expressing two separate genes-of-interest with GFP, RFP, hygromycin, and puromycin selection markers. H929 suspension cells were grown under puromycin and hygromycin selection.

Need a service not listed here? Have questions?
Email us at ***services@systembio.com***



QUICK AND EASY CLONING...REALLY

Fast, easy, and efficient, **Cold Fusion Cloning** (Cat.# MC010B-1) is an excellent choice for any cloning project. Whether you're assembling multiple fragments of DNA or simply adding an insert or gBlock® to a vector, the Cold Fusion Cloning Kit will take you to transformation-ready DNA in a single step—just incubate your DNA fragment(s) with linearized vector for 5 minutes at room temperature and 10 minutes on ice.

- **Fast**—get transformation-ready DNA in as little as twenty minutes
- **Efficient**—obtain >90% positive clones in a typical reaction
- **Easy**—a phosphatase-free and ligation-free system that eliminates the need for specific restriction enzymes
- **Versatile**—add any insert into any site in any vector using manual or automated workflows

*Learn more and order now at **systembio.com/Cold-Fusion-Cloning***

"I must say the Cold Fusion Kit makes it way too easy to design a vector or new construct—it's a really good product."

Hidevaldo B. Machado, Ph.D., UCLA

About System Biosciences

Seeking out novel technologies and tomorrow's hot new research areas, the team at SBI accelerates research by striving to be the first company to develop and commercialize new inventions. From novel genome editing tools to exosome research, expression and imaging vectors, RNAi libraries, and stem cell tools, SBI harnesses today's innovations to drive tomorrow's discoveries.

2438 Embarcadero Way, Palo Alto, CA 94303
Toll Free: 888-266-5066 www.systembio.com

SBI System Biosciences
Harnessing innovation to drive discoveries